QUALITATIVE EFFECTS ASSOCIATED WITH RED PHOSPHORUS SMOKE INHALATION EXPOSURES IN TWO WILDLIFE SPECIES: PRAIRIE DOGS AND ROCK DOVES

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Effects of red phosphorus/butyl rubber (RP/BR) smoke on two wildlife species, prairie dogs and rock doves (pigeons), were evaluated in laboratory range-finding experiments. Prairie dogs exposed to either 2.0, 4.0, or 6.0 mg/L concentrations of smoke showed no mortality within 30 days after one to four 1-h exposure sessions. Rock doves exposed to either 3.0 or 6.0 mg/L concentrations over 1–4 sessions, however, showed 26% mortality within 8 days postexposure. Male rock doves were much more vulnerable to RP/BR smoke effects, with 42% mortality, in contrast to 6% in the females. Assessments indicated affected or lost vocalization capability in both species, abnormal body postures in rock doves, and increased respiratory congestion in prairie dogs postexposure. Neither species showed definite, consistent effects in gross necropsy and histopathology assessments. Only a few of the rock doves in 6.0 mg/L groups showed signs of excess mucus or exudate in their nasal passages and larynges.

INTRODUCTION

Red phosphorous/butyl rubber (RP/BR) generates a dense white smoke screen when burned to conceal military troop movements (Burton et al., 1982). The white smoke consists primarily of finely divided phosphoric and polyphosphoric acid particles, along with trace amounts of carbon monoxide gas. Health effects and risks associated with inhalation of RP/BR smoke are currently being evaluated by the Health Effects Research Division, U. S. Army Medical Research and Development Command Laboratory at Fort Detrick, Frederick, Md.

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Lethal effects of RP/BR combustion products have been evaluated in two investigations. Burton et al. (1982) exposed albino rats to a range of concentrations from 1.5 to 8.5 mg/L. Their data indicated a lethal concentration value (LC50) of 2.46 mg/L for 5 daily 1-h exposure sessions. Necropsy examinations indicated laryngeal and epiglottal injury at exposure levels above 5.0 mg/L. Signs observed in the rats included pulmonary congestion, edema, and hemorrhage. A second study involving albino rats (Aranyi, 1983) indicated an LC50 value of 2.32 mg/L for 5 daily 1-h exposures.

In previous in vitro tests (Aranyi, 1983), rat lung tissue showed reduced resistance to an infectious agent (S-K pneumoniae) after 1-h RP/BR smoke exposures at 0.5 mg/L concentration. More recently, Aranyi et al. (1988) reported that RP/BR smoke produces lung fibrosis in vivo at 1.2 mg/L with 40 h of total exposure in laboratory rats. Rat groups exposed to 0.75 mg/L or higher concentrations for 40 h showed mild to moderate terminal bronchiolar fibrosis in the respiratory tract.

In terms of ecotoxicological effects, some research (Van Voris et al., 1986) has been conducted on various plant species after RP/BR smoke exposures. No published reports are available, however, related to the inhalation effects of this material on wild animals. The potential effect on animal communities adjacent to or on military training installations was one of the primary concerns of the current study. Black-tailed prairie dogs *(Cynomys ludovicianus)* and rock doves *(Columba livia)* were selected as indicator species based on their extensive overlapping ranges throughout the central United States, their availability, and their adaptability to the laboratory environment.

The purpose of this study was to describe the qualitative toxicological effects of RP/BR smoke inhalation exposures in concentration range-finding tests (Shumake et al., 1989). Based on concentration ranges determined in these tests, more refined and quantitative behavioral and physiological effects such as spontaneous activity, startle response, pulmonary function, and blood chemistry were later investigated in these two species (Sterner et al., 1990). (References to trade names for identification do not indicate endorsement by the authors or the federal government.)

MATERIALS AND METHODS

Animals

Black-Tailed Prairie Dogs All prairie dogs were captured at Buckley Air National Guard Base, Aurora, Colo., by dispensing large volumes of soap and water into individual burrows (see Elias et al., 1974). After transport to our laboratory, the animals were dusted with pyrethrum-containing powder to control ectoparasites, weighed, and implanted with a subcutaneous transponder (Identification Devices, Inc., Boulder,

Colo.) for individual identification (Fagerstone and Johns, 1987). During a 14-day acclimation period and throughout the range-finding studies, the prairie dogs were housed individually and were fed Purina Rabbit Checkers ad libitum and fresh cabbage 3 times per week.

Rock Doves A shipment of 122 rock doves was purchased from a local supplier. Birds were captured with cannon nets (Grubb, 1988) in the north Denver area. Upon receipt, the birds were placed in wire mesh outdoor aviary cages and maintained on a diet of Purina Pigeon Checkers, cracked corn, grit, and water. After 13 wk, the doves were moved to an indoor quarantine facility with heat and light provided. Birds were leg-banded with individual identification numbers, weighed, and dusted for ectoparasites. During quarantine and thereafter, the birds were maintained on Purina Pigeon Checkers, and water ad libitum. The rock doves were allowed 14 days of acclimation in a different building before smoke exposure testing was begun.

Inhalation Exposure System

Air Filtration, Humidification, Cooling: RP/BR Smoke Generation; and Inhalation Chambers As shown in Figure 1, laboratory room air was first humidified (Sterner et al., 1991), then passed through an Absolute Filter Unit (Young and Bertke Co., Cincinnati, Ohio). The system air flow rate was regulated by a vacuum unit located just behind a DX-grade coalescent filter unit. This final filter removed over 99% of RP/BR smoke aerosol and associated gases from the chamber exhaust (Holmberg et al., 1985). The air flow rate was held constant within the chamber at 250 L/min throughout all exposures.

The RP/BR extruder-generator system has been previously described in detail (Holberg et al., 1985). The RP/BR product, formulated at the Bio/Organic Analysis Section, Analytical Chemistry Division, Oak Ridge National Laboratory (ORNL), was extruded at a steady rate by a precision metering pump into a glass chamber for controlled combustion to generate specific concentrations of the RP/BR aerosol. The RP/BR smoke-laden air was then cooled by water jackets connected to a refrigerated bath unit (Sterner et al., 1991).

A uniform dispersion of the RP/BR smoke in the stainless steel exposure chamber (Bertke and Young, Cincinnati, Ohio; 0.914 m^3 capacity) had been confirmed previously by uniformity tests (Sterner et al., 1988). The chamber contained 3 stainless steel mesh shelves that each held 4 removable stainless steel animal cages (30 × 30 × 30 cm). The interior of the chamber and the cages were flushed with tap water after each exposure session to minimize surface residues of condensed phosphoric acid mist. The bottom of the chamber contained a drain pipe with valve for water-flushing of smoke residues into the laboratory floor drain.

In a separate room, a 0.914-m^3 stainless steel control chamber system (Bertke and Young, Cincinnati, Ohio) was used to expose separate

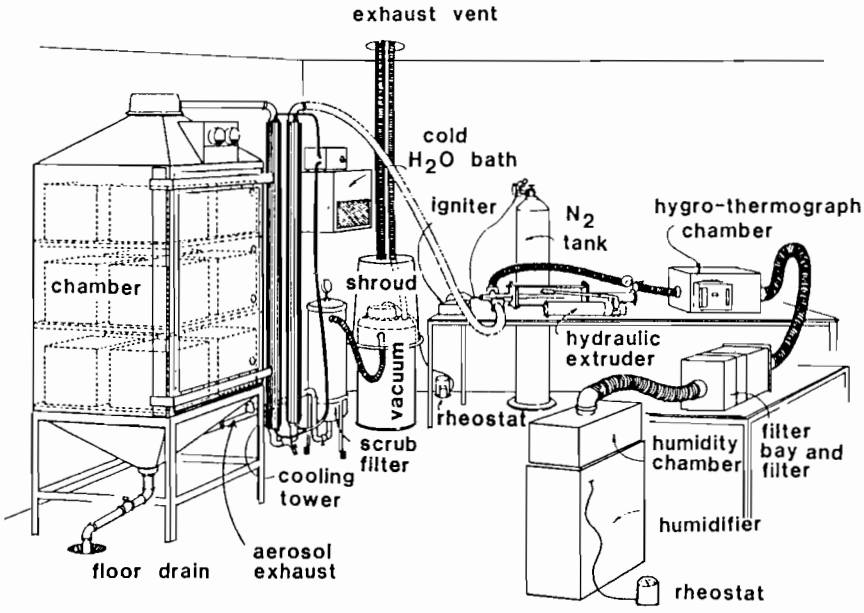

FIGURE 1. Technical illustration of the inhalation chamber system with the RP/BR hydraulic extruder located just ahead of the cooling tower. A precision metering pump was used to slowly add continuous pressure to a hydraulic piston to extrude the pliable RP/BR product. This was ignited to generate a continuous stream of smoke that was added to the filtered air stream and precooled before entering at the apex of the chamber. Airflow rates through the system were controlled by a commercial vacuum operated by a rheostat and monitored via air pressure gauges (from Sterner et al., 1991).

groups of prairie dogs and rock doves to filtered air for equivalent time periods. Components were essentially similar to the smoke exposure system except for the absence of the water jacket, the final filter unit, and the RP/BR product.

Determination of RP/BR Smoke Concentrations Aerosol mass was determined for each exposure session by weighing a filter holder and fiberglass filter before and after exposure to 1 L/min of smoke from the chamber for the entire session. Phosphoric acid concentration was measured using an automatic titration system (Radiometer America, Inc., Cleveland, Ohio) and fiberglass filter sample disks. The determinations produced an index of the total phosphorus content (Burton et al., 1982) for each exposure session. Aerosol opacity was measured with an ORNL Sensor (Higgens et al., 1978; Holmberg et al., 1985). Chart records of the relative opacity of smoke during each exposure session were used to continuously monitor smoke concentration and, in conjunction with the aerosol mass measure, to determine the steady-state concentrations achieved during each session. Particle size was measured in terms of

mass median aerodynamic diameter (MMAD) and geometric standard deviations (σ_g). A Quartz-Crystal-Micro-Balance (QCM) cascade impactor (California Measurements, Inc. Sierra Madre, Calif.) was used to measure particle sizes ranging between 0.10 and 25 μm. Respiratory and contaminant gases (O_2, CO_2, CO, PH_3, C_6H_{14}) were measured for concentration using industrial analyzer tubes (Gastec Inc., Newark, Calif.). Temperatures of the smoke exposure conditions in the chamber were measured at 20-min intervals during exposures; relative humidity was measured at the beginning and end of each exposure using a wet bulb/dry bulb thermometer inserted into the main air exhaust line from the chamber. Time of exposure was based on 60 min of burning of the RP/BR product, but the animals were confined to the exposure chambers for up to 40 min beyond this period to allow adequate exhaust venting of the RP/BR aerosol before transferring animals back to the home cages.

PROCEDURES

Prairie Dog Groups

Experimental Design In the initial study, 48 animals (24 of each sex) were randomly selected from a pool of 110 captured prairie dogs. The animals were then assigned to 8 groups in order to evaluate RP/BR aerosol effects over the range of 2.0–6.0 mg/L with up to 2 successive exposure sessions.

To approximately match body weight ranges for each group, animals of each sex were first rank-ordered according to body weight. Then three arbitrary weight classes were set up, each consisting of eight animals. For the males, these weight classes were light weight (733–911 g), medium weight (916–1019 g), and heavy weight (1029–1189 g). For the females, these respective weight class ranges were: 634–795 g, 803–891 g, and 917–1099 g. Next, one male and one female prairie dog from each of the light, medium, and heavy weight classes (i.e., three males and three females) were randomly assigned to one of the eight treatment groups.

Six of the initial substudy groups received either 2.0, 4.0, or 6.0 mg/L target concentrations of RP/BR-smoke on either 1 or 2 successive exposure sessions. The remaining two groups received one or two exposure sessions to filtered air for comparable durations.

When no pronounced RP/BR smoke exposure effects were observed in this study, additional groups were exposed for more sessions in the second study 4 mo later. In this study, 24 prairie dogs (12 of each sex) from the remaining 62 captured animals were randomly assigned to 4 treatment groups (n = 6/each). The same rank-ordered body weight classing procedure was used to assign animals of each sex to the light, medium, and heavy weight categories. The weight ranges for males were 947–1116 g, 1170–1244 g, and 1279–1516 g. For females, these ranges were 875–933 g, 1012–1054 g, and 1071–1276 g. Again, one male and one female

prairie dog were randomly selected from each of the three weight classes (i.e., three males and three females) and were assigned to one of the four treatment groups.

Two of the groups received either 3 or 4 successive daily exposures to RP/BR-smoke at a target concentration of 6.0 mg/L. The two remaining groups received four successive daily exposures to filtered air for comparable durations. One of the filtered-air groups was given "regular handling" being restrained for respiration checks before and after exercise; the second filtered-air group was given "minimal handling" without these checks and restraint in order to assess any effects associated with the stress of handling.

For both studies, toxicity assessments were conducted with the same experimental paradigm. The design consisted of a 7-day preexposure phase (baseline), a 1- to 4-day exposure phase, and a 30-day postexposure phase.

Toxic Signs and Mortality Assessments Assessments were performed on animals within 24 h postexposure in each group throughout the 3 experimental phases. Animal groups were checked daily for mortalities. The standardized examination for toxic signs required 3–5 min per animal and involved the following sequence of events: (1) visual determination of body posture and coat condition, (2) determination of respiratory congestion with a stethoscope while the animal was resting, (3) measurement of body weight, (4) forced activity (30 cm/s required running speed) for 60 s in an activity wheel, with the animal prodded to run, (5) immediate determination of postexercise respiratory congestion and aggression, and (6) a second determination of body posture effects after exercise. Vocalization effects were monitored and noted throughout each session. Table 1 more fully describes the categories of toxic signs used to evaluate qualitative effects of the RP/BR smoke exposures in prairie dogs. To evaluate the consistency of scoring, 18 prairie dogs were independently rated on the same day during the preexposure phase by 3 different investigators. Interobserver reliability was later assessed by calculating the percent of agreement among ratings.

Necropsy and Histology On day 31 postexposure, all animals were euthanized with sodium pentobarbital (Schering Corp., Kenilworth, N.J.) injected ip. Postmortem necropsy examinations included assessments of the following organs by APHIS Veterinary Services (Denver, Colo.) personnel: nasal passages, trachea, larynx, epiglottis, bronchi, lungs, heart, liver, spleen, and kidneys.

Sections of the nasal passages, bronchi, lungs, and liver, along with the entire trachea, larynx, and epiglottis from each animal, were preserved jointly in specimen jars containing 10% formalin solution. Tissue slide specimens were later prepared and examined for histopathological signs by staff of the Pathobiology Laboratory, Parasitology and Clinical Pathology Section, NVSL (Ames, Iowa).

TABLE 1. List of the Six Toxic Sign/Mortality Categories, plus the Operational Procedures or Definitions Used to Rate Each Prairie Dog During the 7-Day Preexposure, 1- to 4-day Exposure, and 14-Session Postexposure Phase (i.e., Days 1–7, 10, 13, 16, 19, 22, 25, and 28)

Category	Operational procedure/definition
Body posture (rest and postexercise)	The body posture of each prairie dog was rated while at rest in the home cage and after 60 s of mild exercise in a motorized stainless steel activity wheel (100 cm diameter × 20.6 cm wide). The wheel rotated at approximately 30 cm/s during exercise. Abnormal ratings were assigned to animals that remained in a prostrate position with their abdomen against the cage floor or remained "hunched over" with head down. Positive ratings were assigned to prairie dogs that displayed these postures even though prodded gently on the back (3–5 cm above the base of the tail) with a pen.
Respiratory congestion (rest and postexercise)	Respiratory congestion was checked before and after exercise using a stethoscope (Propper Mfg. Co., Long Island City, N.Y.). Abnormal ratings were assigned to prairie dogs that displayed harsh rasping, gurgling, whistling, wheezing, or buzzing sounds from the chest that were associated with movements of the chest or nostrils.
Coat condition	The coat condition of each prairie dog was rated prior to exercise as groomed or ungroomed. Positive ratings for ungroomed were assigned to coats that had matted, wet-looking, gnarled, or raised-up hair patterns. The sign was distinguished from shedding, uneven, and thinning hair.
Aggression	The aggression that each prairie dog displayed toward handlers was scored as high, medium, or low. Positive ratings were assigned to prairie dogs that persisted in biting and growling during the examination.
Vocalization (occurrence and quality)	The occurrence (present, absent) and quality of vocalizations (normal, affected, or lost) were noted for those animals that barked during the examination session. An occurrence of vocalization was recorded if an individual prairie dog was observed emitting sounds while being examined during a daily session. For quality ratings, normal vocalization was a series of 8–20 short chattering-type barks or shrill yips. Affected vocalization was a low pitch and low volume bark/yip, with a raspy (laryngitis-type) quality, similar to severe laryngitis.
Mortality	Death (i.e., lack of respiration and heartbeat) was determined daily for each prairie dog during an initial check of the cages by an investigator (i.e., 0800–0900 MST).

Data Analyses Tabular and graphical descriptive methods were used to evaluate changes in frequencies of the qualitative signs. Gross necropsy and histological data were also treated descriptively. That is, the percentage of tissue specimens that showed a given type of pathology was calculated and compared for RP/BR-smoke exposed versus control groups.

Rock Dove Groups

Experimental Design Rock doves were held in individual galvanized wire mesh cages (51 × 27 × 38 cm) in a separate room with temperature (23 ± 2°C) and light : dark cycle (12 : 12) control. Throughout testing, the birds were fed the same diet as during the quarantine.

A cloacal examination procedure (Miller and Wagner, 1955) was used for determining the sex of each dove. Verification of sex occurred upon gross necropsy at the end of the study period. From the captured doves, 48 birds (24 of each sex) were randomly selected for the study. Body weight ranges of each of eight groups (three males and three females) were approximately matched by a preassignment ranking procedure. The 24 birds for each sex were rank-ordered according to body weight. Then three arbitrary weight classes were set up (each consisting of eight birds). For males, these were light (270–329 g), medium (333–352 g), and heavy (352–389 g). For females, these respective weight class ranges were 277–307 g, 313–334 g, and 335–363 g. Next, one male and one female rock dove from each of the light, medium, and heavy body weight groups (i.e., three of each sex) were randomly assigned to one of the eight treatment groups.

Six of the groups received either 1, 2, or 3 successive daily exposures to RP/BR-smoke target concentrations of 3.0 and 6.0 mg/L. The remaining 2 groups received either 4 successive daily exposures to RP/BR aerosol at the 6.0 mg/L target concentration or 4 daily exposures to filtered air. All exposure sessions were approximately 80 min (60 min of RP/BR burning), with minor variations due to required venting time of the inhalation chamber.

The same experimental paradigm previously described for the prairie dog studies was also used for the rock dove study, and three kinds of toxicity-assessment variables were measured: toxic signs/mortality, gross necropsy, and histopathology.

Toxic Signs and Mortality Assessments Mortality was determined daily throughout all phases of the range-finding study. Toxic sign examinations occurred within 24 h postexposure and required 4–6 min per animal. They consisted of the following sequence: (1) assessment of body posture and plumage condition, (2) rating of aggression and respiratory congestion (at rest), (3) body weight measurement, (4) inducement of 6 flight crossings of the length of a 3.3 × 1.6 × 2.7 m cage, (5) immediate rating of respiratory congestion and aggression after flight exercise, and (6) a

second determination of body posture effects. Vocalization assessments were made throughout these sessions. A more thorough description of the categories of toxic signs used to evaluate effects of RP/BR smoke in the rock doves is found in Table 2.

Necropsy and Histology On day 31 postexposure, all doves were euthanized with sodium pentobarbital injected ip for postmortem analyses. The gross necropsy and histology procedures essentially paralleled those described for the prairie dog tests.

Data Analyses Toxic sign, mortality, gross necropsy, and histopathology data were analyzed using descriptive statistics, a chi-square test, and graphical illustrations. Preexposure baseline and control group data were used for comparing changes in frequencies of the abnormal (positive) signs. Toxic sign data were presented in tabular form for comparison of the percentage of observations for each measure that were positive, based on all surviving rock doves in each group.

RESULTS

Black-Tailed Prairie Dogs

Aerosol Monitoring Steady-state concentrations of the RP/BR smoke during prairie dog exposure measured close to target levels with median and ranges of 1.8 (1.3–1.8), 4.3 (4.3–4.4), and 5.9 (5.6–9.4) mg/L for the 2.0, 4.0, and 6.0 mg/L targets, respectively. Levels of phosphoric acid for filter disk samples collected at 1.0 L/min were 78.0 (71.2–85.9), 195.8 (182.8–198.2), and 257.5 (234.8–283.9) mg for the 3 target concentrations, respectively. No phosphoric acid levels were detected from the control chamber samples. The percentage of RP/BR aerosol mass attributable to phosphoric acid ranged in median values from 70.5 to 76 (total range 69–76) in agreement with ranges found by others (Moneyhun et al., 1988). Mass median aerodynamic diameters (MMAD) and ranges of aerosols were 0.64 (0.62–0.66) and 0.88 (0.82–0.94) μm for the 2.0 and 4.0 mg/L target concentration exposures. Geometric standard deviations for these respective concentrations had ranges of 1.51–1.54 and 1.34–1.49. The 6.0 mg/L exposure MMADs were not determined due to rapid saturation of the QCM detector crystals at these levels. Median levels of oxygen (18–22%), carbon dioxide (605–968 ppm), carbon monoxide (8.5–24 ppm), and phosphine gases (0–0.2 ppm) were found to be within safe and acceptable ranges throughout the smoke exposures. For hexane, however, at the 6.0 mg/L concentration exposure level, the median value of 54 ppm was fairly high. Later quality assurance evaluations (Moneyhun et al., 1988) using gas chromatography indicated that this was probably an anomaly of the analyzer tube measurement method and most likely due to interactions of other gases in the RP/BR smoke. For all exposures, in-chamber temperature ranged from 19 to 25°C, relative humidity ranged from 46 to 67%, and total enclosure confinement durations ranged from

TABLE 2. List of the Six Toxic Sign/Mortality Categories, plus the Operational Procedures or Definitions Used to Rate Each Rock Dove During the 7-Day Preexposure, 1- to 4-day Exposure, and 14-Session Postexposure Phase (i.e., Days 1-7, 10, 13, 16, 19, 22, 25, and 28)

Category	Operational procedure/definition
Body posture (rest and postexercise)	The body posture of each rock dove was rated while at rest in the home cage and after 60 s of forced flight. For exercise, each dove was prodded with a 1-m wooden dowel rod to make 6 flights of approximately 3.3 m from perch to perch within a 3.3 × 1.6 × 2.6 m fiberglass and wire mesh (1.3 cm^3) flight cage. Abnormal ratings were assigned to birds that were listing forward with head and chest "hunched over."
Respiratory congestion (rest and postexercise)	Each dove was checked for signs of respiratory congestion before and after exercise. Abnormal ratings were assigned to birds that displayed "parted (open) beak" or audible breathing sounds of gasping, rasping, wheezing, or sneezing. Audible respiratory sounds were determined by holding the dove's head and chest within a few centimeters of the investigator's ear; a stethoscope was not used for this measurement.
Plumage condition	The plumage condition of each rock dove was rated as kempt or unkempt. Unkempt ratings were assigned to birds with feathers that were ruffled or dull in appearance.
Aggression	The aggression that each rock dove displayed toward handlers was scored as high, medium, or low. Positive ratings were assigned to doves that persisted in batting the handler's hand with the wings or pecking at the handler when removed from the home cage and examined.
Vocalization (occurrence and quality)	The occurrence (present, absent) and quality of vocalizations (normal, affected or lost) were noted for those birds that cooed during the examination session. An occurrence of vocalization was recorded as present if an individual dove was observed emitting sounds during an examination session. Ratings of quality were assigned as follows: normal—a low-pitch (guttural) cooing sound; affected—a broken or raspy cooing sound; and lost—beak movements indicating unsuccessful attempts to coo, with only the sound of rushing air or gasping emitted.
Mortality	Death (i.e., lack of respiration and heartbeat) was determined daily for each rock dove during an initial check of the cages by an investigator (i.e., 0800-0900 MST).

74 to 100 min with chamber venting accounting for 14–40 min after the RP/BR product had been extinguished.

Mortality and Toxic Signs No deaths were observed in any of the 72 prairie dogs under each of the substudies, indicating that these animals can readily survive high-level multiple exposures normally lethal to laboratory rats (Aranyi, 1983).

In an assessment of interobserver reliability of the toxic sign category scores, the 3 investigators showed 95–100% agreement for body posture, respiratory congestion, coat condition, and affected vocalization. Categories with poorest agreement were attempted vocalization (87.5%) and aggressive display (81.3%).

Toxic sign data from the two substudies have been combined for comparison of frequencies in Table 3. Two main effects were in evidence that reflected changes in vocalization and respiratory congestion. Lost or affected vocalization was a main sign associated with all RP/BR aerosol groups that were exposed to 6.0 mg/L levels of smoke, especially those that received 4 daily exposures. Half (11 : 22) of the vocalization counts for these animals were in the affected or lost category during the first week postexposure (Post 1). Respiratory congestion was also highest in frequency in this group during this period. Respiratory congestion increased in frequency counts in all other 6.0 mg/L groups and also in the 2-day exposure, 4.0 mg/L group. All other qualitative toxic sign categories either remained unchanged from the preexposure period or were not substantially different from the 0.0 mg/L (control) group frequencies.

Necropsies and Histopathology In necropsy examinations performed on the 72 animals 30 days postexposure, the only indication of abnormality associated with RP/BR smoke treatments was an increase in the incidence of severe lung congestion in the 6.0 mg/L groups. However, the increase was quite small when the 24 filtered-air exposed animals (12.5%) were compared to the 24 animals previously exposed to the 6.0 mg/L concentration of RP/BR aerosol (20.8%).

Histopathological effects were also marginal. The percentage of interstitial pneumonia (IP) signs in lung tissues were similar for RP/BR smoke versus filtered-air exposed animals (i.e., 15 vs. 13%, respectively). This indicated that IP is naturally present in 10–15% of prairie dogs, and that RP/BR smoke produced no increase in the incidence of this pathology. However, intracytoplasmic inclusions within the ganglionic cells of the larynx were observed in several animals, suggesting a viral infection, but this could not be confirmed by electron microscopic examination. The incidence of this pathology was 64% versus 79% in RP/BR smoke- versus filtered-air-exposed animals. Thus, these data were not consistent with the increased frequency of affected or lost vocalization signs shown by the RP/BR smoke-exposed groups.

For liver specimens, the incidences of "mild, moderate, or severe, diffuse hepatocellular swelling and degeneration with cholestasis" were

TABLE 3. Frequency of Positive Toxic Signs in Each Category over 7-Day Blocks for Each Tested Prairie Dog Group Exposed to 4 Levels of Red Phosphorus/Butyl Rubber Smoke for 1–4 Daily Exposure Sessions

	Exposed 1 day			Exposed 2 days			Exposed 3 days			Exposed 4 days[d]		
Sign	Pre[a]	Post 1[b]	Post 2[c]	Pre	Post 1	Post 2	Pre	Post 1	Post 2	Pre	Post 1	Post 2
					6.0 mg/L Target concentration							
Body posture (rest)	2	1	4	0	3	0	0	0	0	0	0	0
Body posture (exercise)	1	0	0	0	2	0	0	0	0	0	1	1
Respiratory congestion (rest)	0	1	3	0	0	3	0	3	3	0	14*	6*
Respiratory congestion (exercise)	0	2	4	0	0	7*	0	9*	5*	0	13*	8
Ungroomed coat	0	0	4	0	0	1	0	0	0	0	0	0
Aggressive responses	4	4	1	4	2	10	8	5	1	1	4	2
Vocalizations	25	20	18	28	23	15	9	10	7	20	23	8
Normal	24	18	12	28	13	12	9	10	4	20	12	3
Affected	1	2	6*	0	6*	3	0	0	3	0	2	3
Lost	0	0	0	0	4*	0	0	0	0	0	9*	2
					4.0 mg/L Target concentration							
Body posture (rest)	0	0	0	0	0	0						
Body posture (exercise)	0	0	0	0	0	0						
Respiratory congestion (rest)	0	1	0	0	1	1						
Respiratory congestion (exercise)	0	1	1	0	4*	0						
Ungroomed coat	1	0	1	0	0	0						
Aggressive responses	0	2	0	0	2	1						
Vocalizations	23	14	16	22	9	5						
Normal	23	13	15	22	7	4						
Affected	0	1	1	0	2	1						
Lost	0	0	0	0	0	0						

2.0 mg/L Target concentration									
Body posture (rest)	0	0	0	2	1	0			
Body posture (exercise)	0	0	0	0	0	0			
Respiratory congestion (rest)	0	0	1	0	0	0			
Respiratory congestion (exercise)	0	0	1	0	0	0			
Ungroomed coat	0	0	1	0	0	0			
Aggressive responses	0	1	0	8	8	5			
Vocalizations	23	8	7	21	17	13			
Normal	23	8	6	21	15	11			
Affected	0	0	1	0	2	2			
Lost	0	0	0	0	0	0			
0.0 mg/L Target concentration									
Body posture (rest)	0	1	0	2	4	0	0	0	0
Body posture (exercise)	0	0	0	0	3	0	0	0	0
Respiratory congestion (rest)	0	0	0	0	0	3	1	2	2
Respiratory congestion (exercise)	0	0	0	0	1	0	0	2	2
Ungroomed coat	2	0	0	0	0	0	0	0	0
Aggressive responses	2	0	0	2	3	3	9	11	10
Vocalizations	25	21	13	16	9	3	24	32	19
Normal	24	21	13	16	7	3	23	32	19
Affected	1	0	0	0	2	0	1	0	0
Lost	0	0	0	0	0	0	0	0	0

Note. Increased sign frequencies are shown by asterisk and were detected by comparison with prevalues as well as control group (0.0 mg) values.

[a]Refers to a 7-day period prior to RP/BR-aerosol or filtered-air exposure. Data are the total recorded counts for each toxic sign category for six prairie dogs during this period.

[b]Refers to the first 7-day period beginning on the day after the last RP/BR-aerosol or filtered-air exposure. Data are the total counts for each toxic sign category for six prairie dogs during this period.

[c]Refers to the day 10 through day 28 period: data are taken every third day (i.e., days 10, 13, 16, 19, 22, 25, and 28) and are the total recorded counts for each toxic sign category for 6 prairie dogs during this period.

[d]Data presented only for "regular handling" control group (0.0 mg/L) animals. "Minimal handling" animals showed a similar frequency pattern for toxic sign categories.

98 and 88% of RP/BR-aerosol- and filtered-air-exposed animals, respectively. "Minimal to mild, multifocal, hepatocyte necrosis" was found in 10 versus 4%, respectively, of animals in these 2 exposure conditions.

The percentage of "mild or moderate multifocal, epithelial ulcerations and necrosis" for tracheal specimens was 58 versus 54 between these same pools of prairie dogs. Again, these results indicated no difference in incidences of pathology in tracheal tissues.

The percentage incidence of "mild, moderate or severe multifocal hemorrhage and necrosis" in lung tissues was 87.5 versus 62.5 between "pooled" RP/BR- and filtered-air-exposed specimens, respectively. In addition, severe hemorrhage was found in 23% of lung tissues for RP/BR-aerosol exposed animals as compared to 8% of lung samples from control animals. Although these differences are noteworthy, there is still no clear evidence that any of the hemorrhages resulted from RP/BR-aerosol exposure. More likely, small exertive movements or the position of the prairie dogs during euthanasia and prior to death could have caused such effects.

Rock Doves

Aerosol Monitoring For rock dove exposures, steady-state concentrations of the RP/BR smoke attained median and range values of 3.3 (2.9–3.4) and 5.9 (5.3–6.3) for the 3.0 and 6.0 mg/L targets respectively. Phosphoric acid levels were measured at 144.8 (131.5–159.1) mg and 255.5 (232.4–280.5) mg for the 2 concentrations, respectively. The percentage of RP/BR aerosol mass attributable to phosphoric acid ranged in median values from 70.5 to 76 (total range 69–76). These values were within the ranges expected based on previous studies (Sterner et al., 1988; Moneyhun et al., 1988). The median and range of MMADs values for the 3.0 mg/L concentration were 0.82 (0.68–0.89) μm (6.0 mg/L samples were not measured due to crystal saturation of the QCM). Geometric standard deviations for the 3.0 mg/L concentration ranged from 1.32 to 1.54. Median levels of oxygen (19–19.6%), carbon dioxide (448–726 ppm), carbon monoxide (9.1–24.8 ppm), and phosphine gas (not detected–0.1 ppm) were found to be within safe ranges throughout the exposures. Again, however, at the 3.0 and 6.0 mg/L concentration levels the median hexane values were high at 18.1 and 60.5 ppm, respectively. As previously indicated, these high readings were probably anomalous, as indicated by separate chamber readings using gas chromatography. For all exposures, in-chamber temperatures ranged from 18 to 22°C, relative humidity ranged from 48 to 64%, and total enclosure confinement ranged from 73 to 87 min with chamber venting accounting for 13–27 min after the RP/BR product was extinguished.

Mortality and Toxic Signs Overall mortality results have been tabulated and are shown in Table 4. None of the 6 rock doves in the filtered-air-exposed (control) group died in the 30-day postexposure period. As

TABLE 4. Rock Dove Mortality Data

Target RP/BR aerosol concentration	Number of daily exposure sessions	Male morality ratio[b]	Postexposure days until death	Female mortality ratio[b]	Postexposure days until death	Total mortality ratio[b]
0.0	4	0 : 3	—	0 : 3	—	0 : 6
3.0	1	0 : 3	—	0 : 3	—	0 : 6
3.0	2	0 : 3	—	0 : 3	—	0 : 6
3.0[a]	3	1 : 4	5	0 : 2	—	1 : 6
6.0[a]	1	2 : 4	(7, 8)	0 : 2	—	2 : 6
6.0	2	3 : 3	(5, 6, 6)	0 : 3	—	3 : 6
6.0	3	2 : 3	(5, 6)	1 : 3	5	3 : 6
6.0[a]	4	2 : 4	(1, 5)	0 : 2	—	2 : 6
Ratio totals		10 : 27		1 : 21		11 : 48

[a]One male in each of these groups was initially missexed by the cloacal examination method at the start of the study. They were determined to be males upon necropsy at the end of the postexposure period.

[b]Number of deaths : total number of birds tested.

indicated, 2–3 animals per group died after RP/BR-smoke exposures at the 6.0 mg/L target concentration over 1–4 daily sessions. One animal died in the 3.0 mg/L group given 3 exposure sessions.

The data indicated that male rock doves had a much higher percentage of mortality associated with RP/BR aerosol than females. Comparing only the 7 RP/BR-aerosol-exposed groups, 10 of 24 males (41.7%) versus 1 of 18 females (5.6%) died within 5.4 ± 1.7 days postexposure. Based on the number of deaths in these male versus female groups, the chi-square value (5.181, df = 1, $p < .05$) was significant. Also, a significant chi-square value (5.025, df = 1, $p < .05$) was obtained for mortality of male versus female rock doves exposed to only the 6.0 mg/L smoke concentration for 1–4 sessions.

Toxic signs data were compared based on the percentage of total observations on surviving doves in each group that were positive for toxic signs. The respiratory congestion sign category was later found to be biased toward positive effects after the exposure period for all groups.

Stethoscopic detection of congestion in doves proved to be difficult due to the predominant heartbeat sounds from the chest area. The unaided ears and eyes of the observers were used to detect raspy, gurgling breathing sounds or accentuated parted-beak gasping responses.

The toxic sign results are presented in Table 5; three main findings characterized the data. First, all 7 of the RP/BR smoke groups showed a low level of affected vocalization ranging from 2.2 to 12.5%. None of the filtered-air-exposed doves showed this sign pre- or post-exposure. Second, 6 of 7 RP/BR smoke groups showed an increased percentage of the affected body posture signs during wk 1 postexposure (Post 1). Third, the

TABLE 5. Percentage[a] of Positive Toxic Signs in Each Category Based on the Total Number of Observations in 7-Session Blocks for 8 Rock Dove Groups Before and After Exposure

	Exposed 1 day			Exposed 2 days			Exposed 3 days			Exposed 4 days		
Sign	Pre[b]	Post 1[c]	Post 2[d]	Pre	Post 1	Post 2	Pre	Post 1	Post 2	Pre	Post 1	Post 2
	6.0 mg/L Target concentration											
Body posture (rest)	0	0	0	4.5	26.8*	4.7	0	3.5*	0	0	3.5*	0
Body posture (exercise)	0	0	2.8*	0	24.5*	9.4*	0	5.8*	0	0	2.8*	0
Respiratory congestion (rest)	0	4.7	10.0	0	38.7*	4.7	0	15.4	14.1	0	17.8	17.8
Respiratory congestion (exercise)	0	23.7	31.4	4.7	70.8*	28.2	4.7	47.4	61.5	4.7	38.5	25.0
Plumage unkempt	2.2	4.7	3.5	0	0	0	0	0	0	0	0	3.5*
Aggressive responses	4.5	0	17.1	4.5	10.2	23.5	23.4	23.4	23.5	0	0	0
Vocalizations	21.2	21.1	27.8	35.4	45.5	85.4	30.3	20.0	51.8	23.4	32.8	32.1
Normal	21.2	14.0	24.2	35.4	29.8	84.4	30.3	17.7	51.8	23.4	26.4	28.5
Affected	0	7.1*	3.5*	0	12.5*	0	0	2.2*	0	0	6.4*	3.5*
Lost	0	0	0	0	2.8*	0	0	0	0	0	0	0
	3.0 mg/L Target concentration									0.0 mg/L Target concentration		
Body posture (rest)	0	2.2*	0	2.2	4.5*	2.2	0	2.2*	0	0	0	0
Body posture (exercise)	0	0	0	0	2.2*	2.2*	0	2.2*	0	0	0	0
Respiratory congestion (rest)	0	11.7	4.5	0	16.4	11.7	0	17.4	17.1	0	14.1	2.2
Respiratory congestion (exercise)	7.1	52.0	52.2	0	54.4	52.1	7.0	42.7	31.4	0	44.8	54.5
Plumage unkempt	0	0	0	0	0	0	0	2.2	0	0	0	0
Aggressive responses	18.7	35.4	38.0	7.0	18.9	25.7	6.8	9.2	8.5	5.5	2.2	16.4
Vocalizations	40.1	61.5	52.0	37.7	66.2	63.8	21.1	25.0	8.5	28.4	40.1	47.4
Normal	40.1	52.1	49.7	37.7	54.4	61.5	21.1	19.7	8.5	28.4	40.1	47.4
Affected	0	9.4*	2.2*	0	11.5*	2.2*	0	5.1*	0	0	0	0
Lost	0	0	0	0	0	0	0	0	0	0	0	0

Note. Individual groups received RP/BR target concentrations of 0.0, 3.0, or 6.0 mg/L for 1 to 4 successive daily exposure sessions. Increased toxic sign percentages (*) were detected by comparison with individual group prevalues as well as control group (0.0 mg/L) values.

[a]Because a few doves died within 1–8 days after RP/BR aerosol exposure, a percentage of the total number of observations made on the surviving rock doves on each 7-session block that were scored positive was used in place of frequency counts for each toxic sign category. This transformation has the effect of removing potential low count biases when doves died postexposure in certain groups.

[b]Refers to a 7-day period prior to RP/BR-aerosol or filtered air exposure.

[c]Refers to the first 7-day period beginning on the day after the last RP/BR aerosol or filtered air exposure.

[d]Refers to the day 10 through day 28 period with the day after the last RP/BR aerosol or filtered air exposure as the starting date (i.e., the 7 days include days 10, 13, 16, 19, 22, 25, and 28).

6.0 mg/L group, given 2 exposure sessions, was the most severely affected, and this was most consistently shown in the body posture and respiratory congestion sign categories. The percentages of observations in doves in this group that were positive for the body posture sign were 26.8 and 24.5 for rest versus exercise, respectively, during the Post 1 period. The highest mortality rate (50%) was also noted for this group.

Necropsies and Histopathology The postmortem examinations were conducted on all 48 rock doves 30 days after the final RP/BR-aerosol or filtered-air exposures. The 3.0 mg/L aerosol exposure groups showed no increase in the incidence of lung tissue abnormalities. However, 2 doves in the group given 1 exposure at 3.0 mg/L had excess laryngeal mucus, and 1 dove in this group had a large amount of exudate in the bronchi. Another bird in the 3 mg/L, 3-exposure group had excessive exudate in both the larynx and in the epiglottis. For rock doves in the 6.0 mg/L, 3- and 4- exposure groups, there was a slightly increased frequency of mucus or exudate in the nasal passages and larynges. Bronchi were reported as showing one instance each of excess mucus, a large quantity of catarrhal exudate, edemitis, and swelling.

Histopathology examinations of tissue samples revealed several instances of pathology, but these were nonspecific for RP/BR-smoke exposures. The pathology included tracheal lesions, fibrosis, lung hemorrhage, and pneumoconiosis.

More specifically, no significant lesions were found in the nasal turbinates. Also, no significant lesions were found in tissue sections of epiglottis from control and RP/BR aerosol-exposed rock doves. Lymphocytic inflammation was found in sections of larynx from one control and one RP/BR smoke-exposed rock dove. No significant lesions were found in sections of trachea from control birds. Lesions in sections of trachea from RP/BR smoke-exposed birds included lymphocytic inflammation (two animals), hemorrhage (one animal), and fibrosis (one animal). These lesions were thus considered to be nonspecific. Hemorrhages were found in lung sections from 4 animals exposed to filtered air and in 15 RP/BR-aerosol-exposed animals. These incidences of hemorrhages could have been related to the euthanasia procedure. Pulmonary congestion and edema could have also been related to the euthanasia method or to hypostatic changes that occurred after euthanasia. Pneumoconiosis, a lesion found in 3 control animals and 12 of the animals that were RP/BR aerosol exposed, was regarded as an incidental finding. Perivascular lymphocyte infiltrates were found in lung sections of two exposed rock doves. This lesion was also considered to be nonspecific.

Lesions in the liver included periportal inflammation, a nonspecific lesion usually associated with a previous bacterial infection. Hepatic congestion was probably related to the method of euthanasia. Cholestasis was found in liver tissue sections from five exposed rock doves, but this was considered to be nonspecific pathology also. Periportal degenera-

tion and necrosis were found in liver sections from five other RP/BR aerosol-exposed groups but were not related to exposure level. The etiology of these effects remain unknown. In summary, no lesions attributable to RP/BR aerosol exposures were found in the examined tissue samples.

DISCUSSION AND CONCLUSIONS

Main Findings

For the target concentration range of 2.0–6.0 mg/L and up to 4 repeated exposures, no prairie dogs died in the 30-day postexposure period. In contrast, 11 of 42 rock doves exposed to similar levels died within 5.4 ± 1.7 days postexposure. Of these, only 1 of 18 died at the 3.0 mg/L RP/BR aerosol level, but 10 of 24 died at the 6.0 mg/L level. In addition there was a significant sex difference based on this preliminary range-finding study. RP/BR aerosol exposures were lethal to 10 of 24 males (42%) but only to 1 of 18 females (6%).

Toxic sign results were partially similar for these two species. Vocalization effects in prairie dogs were detected in those groups given 6.0 mg/L exposures, and these sometimes continued throughout the 30-day postexposure periods. Vocalization effects in rock doves were exhibited to a minor degree in all RP/BR-aerosol-exposed groups for the first week after exposure. Respiratory congestion was found to be a sign associated with multiple exposures to 6.0 mg/L levels in the prairie dog groups. This sign, however, could not be reliably assessed in most dove groups. Six of seven dove groups showed body posture changes postexposure. The rock dove group given 2 exposures at the 6.0 mg/L level showed the highest frequency of all toxic signs as well as the highest mortality rate.

Necropsy data yielded no strong, consistent effects in either species. The only notable increase in frequency of abnormalities was excessive mucus or exudate in the nasal passages and in the larynges of those rock doves exposed to the 6.0 mg/L level for 3 and 4 sessions. This could have been an indication of reduced ciliary motility, as has been previously indicated for sulfuric acid aerosol exposure (Phalen, 1984), as well as depressed immunological responses (Aranyi et al., 1988) associated with RP/BR smoke exposures. Histopathology data indicated no specific pathology that correlated with RP/BR aerosol exposures.

Interspecies Comparisons

Mortality data indicated that both species have higher LC_{50} values for RP/BR aerosol than do Sprague-Dawley albino rats. Burton et al. (1982) and Aranyi (1983) reported data in close agreement that yielded LC_{50} values of 2.46 and 2.32 mg/L for laboratory rats given 5 daily 1-h exposure sessions. Our data indicated that a steady-state concentration of 5.9 mg/L for 4 daily 1-h sessions did not result in deaths of any of the 6 prairie

dogs in this group. As a limited comparison, the data also indicated that a steady-state concentration of 5.9 mg/L RP/BR aerosol exposure for 4 daily 1-h sessions resulted in deaths of one-third of the 6 rock doves in this group.

Aranyi (1983) reported that no observable clinical signs (other than occasional eye-crusting) were shown by albino rats after exposures to RP/BR aerosols at near lethal levels. As previously indicated, respiratory congestion and affected or lost vocalization signs were detectable in prairie dogs after exposure to the 6.0 mg/L target concentration levels. Rock doves exhibited affected vocalization and body posture signs postexposure.

Burton et al. (1982) reported epiglottal and laryngeal ulcers, as well as pulmonary congestion, edema, and hemorrhages, in albino rats exposed to 3.1–8.5 mg/L doses of RP/BR smoke. Our necropsy results indicated a minor degree of lung congestion in some animals as evidenced by excess mucus or exudate in the nasal turbinates and larynges of rock doves 30 days postexposure. Aranyi (1983) reported no incidences of gross pathology in rats exposed to 0.5 mg/L RP/BR smoke for up to 3.5 h.

Our histopathology data also indicated no apparent effects in RP/BR aerosol-exposed prairie dog groups. The Burton et al. (1982) study on albino rats, in contrast, reported an increased incidence of lung hemorrhaging. Histopathological results reported by Aranyi et al. (1988) included terminal bronchiolar fibrosis at levels as low as 0.18 mg/L RP/BR aerosol exposure after 13 wk at 2.25 h/day and 4 days/wk. They found no treatment-specific changes in any tissues examined outside the respiratory tract.

Ecotoxicological Implications and Future Studies

RP/BR smoke concentrations vary greatly under actual field conditions but have been estimated to be between 0.025 and 0.25 mg/L at 2.5 m above the ground, with levels exceeding 0.10 mg/L for less than 1 min after ignition (Garvey et al., 1981). Mass concentrations of RP/BR aerosol at 0.03–0.10 mg/L can obscure equipment at distances of 20–30 ft (Aranyi et al., 1988). The 1.8–5.9 mg/L steady-state 1-h exposures in this study, of course, far exceeded these estimated operational field ranges.

In terms of exposure duration, one of the main uncontrolled factors in the field would be animal movement. Quite likely, prairie dogs would return to their burrows and rock doves would fly out of the smoke-laden areas, reducing the dosage levels of RP/BR smoke material inhaled. Results of the current study should thus be regarded as a preliminary identification of a few avian and mammalian response systems that may be sensitive to RP/BR smoke inhalation exposure effects. The relatively high exposure levels were selected to determine potentially lethal ranges and to define a safe, sublethal range for use in later, more refined studies involving general activity, startle response effects, pulmonary function,

and hematology. Other variables that could be more closely examined in future studies include quantitative and qualitative vocalization effects, species differences, and the sex difference based on mortality.

A more refined analysis of toxic effects produced by RP/BR aerosol exposure would probably involve a more extensive prescreening process to detect latent or active respiratory disease pathology in the two species using blood or tissue sample biopsies. This procedure could reduce variation in the air-control exposed animals, which, in turn, would increase the likelihood of detecting RP/BR aerosol effects.

In addition, other wildlife species with higher metabolic rates and higher respiration rates (e.g., shrews, voles) would probably be more sensitive to RP/BR smoke inhalation effects. Laboratory studies in these other indicator species would be needed to evaluate the toxic signs associated with prolonged and repeated field-level exposures to RP/BR smoke.

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